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Seroprevalence of GB Virus C and Persistence of RNA and Antibody

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Exposure to GB virus C (GBV-C) was determined in several U.S. populations by both reverse-transcription-polymerase chain reaction (RT-PCR) and by an enzyme linked immunosorbent assay (ELISA) for antibodies to mammalian cell-expressed GBV-C envelope protein, E2 (GBV-C E2). Most individuals exposed to GBV-C were either RNA positive/ELISA negative or ELISA positive/RNA negative. Exposure, therefore, was measured as the sum of GBV-C RNA positive and GBV-C E2 antibody positive specimens, and was higher in commercial plasmapheresis donors (40.5%) than in volunteer blood donors (5.5%). In intravenous drug users (IVDUs), GBV-C exposure was 89.2%. Serial bleed specimens tested for GBV-C RNA indicate that some patients remain viremic for at least 3 years and fail to produce detectable antibodies to GBV-C E2. In other exposed individuals who tested negative for GBV-C RNA, antibodies to E2 appear to be similarly long-lived (greater than 3 years) with a fairly constant titer (ranging in reciprocal endpoint dilution from 336 to 21,504). Since the detection of GBV-C RNA and GBV-C E2 antibody are mutually exclusive in most exposed individuals, studies pertaining to incidence and prevalence of GBV-C infection require both antibody and nucleic acid detection. ***J. Med. Virol. 53:167–173, 1997.*** © 1997 Wiley-Liss, Inc.

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INTRODUCTION

GB virus C (GBV-C) is a recently identified human virus consisting of a single-stranded, positive sense RNA genome, approximately 10 kb in length and tentatively classified as belonging to the family *Flaviviridae* [Simons et al., 1995a; Leary et al., 1996a]. Other viruses that are most closely related to GBV-C include hepatitis C virus (HCV), GB virus A (GBV-A), and GB virus B (GBV-B) [Schlauder et al., 1995; Simons et al., 1995a; Muerhoff et al., 1995; Leary et al., 1996a]. Hepatitis G virus (HGV) [Linnen et al., 1996] is actually a different isolate of GBV-C [Zuckerman., 1996; Muerhoff et al., 1996]. GBV-C RNA has been detected in the serum of intravenous drug users (IVDUs), volunteer and commercial blood donors, and patients with cryptogenic hepatitis [Simons et al., 1995b; Leary et al., 1996a; Linnen et al., 1996; Dawson et al., 1996; Schleicher et al., 1996]

Studies on GBV-C prevalence using enzyme-linked immunosorbent assays (ELISAs) employing procaryotically expressed regions of the genome indicate that less than 25% of RNA positive individuals have antibodies to any of these proteins [Dawson et al., 1996]. However, more recent studies using a recombinant envelope protein E2, from GBV-C (GBV-C E2) expressed in mammalian cells indicate that antibody to GBV-C E2 appears in patients who acquired GBV-C through transfusion [Pilot-Matias et al., 1996b; Dille et al., 1997]. The clinical course seen in several of these patients shows development of an antibody response to GBV-C E2 coinciding with the loss of viremia as measured by reverse-transcription-polymerase chain reaction (RT-PCR) [Pilot-Matias et al., 1996b; Dille et al., 1997]. Preliminary data indicate that most individuals exposed to GBV-C are either GBV-C RNA positive/GBV-C E2 antibody negative or GBV-C E2 antibody positive/GBV-C RNA negative [Pilot-Matias et al.,

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1996b; Dille et al., 1997]. Thus, in order to determine exposure to this virus in a population, both GBV-C RNA and GBV-C E2 antibody determinations need to be performed. Although recent data indicates that some individuals remain viremic for many years [Linnen et al., 1996; Dawson et al., 1996; Masuko et al.,1996; Alter et al., 1997], there is no available data on the antibody status of those specimens.

In the present report, prevalence studies were conducted by undertaking both RT-PCR for GBV-C RNA and GBV-C E2 antibody testing on each specimen. In addition, serial specimens were obtained from a number of individuals to determine if their GBV-C status changes over a 1–3 year period. These data demonstrate that GBV-C exposure is more common than indicated by GBV-C RNA testing alone, and that in most cases the GBV-C status in a given individual remains relatively constant over time.

MATERIALS AND METHODS

Reverse-Transcription–Polymerase Chain Reaction (RT-PCR)

Samples were tested for GBV-C RNA by either RT-PCR as previously described [Leary et al., 1996b; Muerhoff et al., 1996; Dawson et al., 1996], or by the reverse transcription-polymerase chain reaction/oligomer hybridization (RT-PCR/OH) assay [Leary et al., 1997]. RT-PCR was carried out using two sets of primers, one reaction utilizing degenerate primers from the conserved helicase domain of NS3 [Leary et al., 1996b] and the second utilizing primers from the 5′-untranslated region [Muerhoff et al., 1996]. The RT-PCR/OH assay was run as previously described [Leary et al., 1997] with detection using an automated IMx detection system (Abbott Laboratories, North Chicago, IL). Overall, 98% of samples evaluated by both methods gave identical results suggesting that either method can be used to determine GBV-C RNA status in serum and plasma specimens [Leary et al., 1997]. RT-PCR for HCV RNA was determined as described previously [Simons et al., 1995b].

Endpoint Titration of RNA

RNA was extracted from 25 ul serum using the Qiamp HCV Kit (Qiagen Inc., Chatsworth, CA) according to manufacturer's instructions. Serial ten-fold dilutions were carried out on the extracted RNA using DEPC treated water (Biotecx Laboratories, Houston, TX) and each dilution was tested for GBV-C RNA by RT-PCR/OH. Samples with a count rate of 100 or greater were considered positive for GBV-C RNA.

Enzyme-Linked Immunoassay for GBV-C E2 Antibody (ELISA)

An ELISA was developed using mammalian cell-expressed GBV-C E2 [Surowy et al., 1997] as previously described [Dille et al., 1997]. Briefly, purified GBV-C E2 antigen was coated onto 1/4″ polystyrene beads at a concentration of 5 ug/ml. The beads were incubated with sera or plasma diluted 1:336 in specimen dilution buffer (SpD) containing protein stabilizers. After incubation, the bound antibody was detected with horseradish peroxidase-labeled goat anti-human IgG (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD). Colorimetric determination was made using 0.3% o-phenylenediamine-2-HCl in 0.1 M citrate buffer, pH 5.5, containing 0.02% H_2O_2 (OPD) (Abbott Laboratories, North Chicago, IL). The cutoff value was determined as the sum of the population mean + 9 standard deviations. Specimens with signal to negative control (S/N) ratios of 10 or greater were considered reactive for antibody to GBV-C E2. Reciprocal endpoint dilution titer was established by testing specimens which were serially diluted in negative human plasma. The resulting reciprocal endpoint dilution is reported as the last dilution that gave a signal to negative control ratio of 10 or greater multiplied by the assay dilution factor of 336.

Radioimmunoprecipitation Assay (RIPA)

A radioimmunoprecipitation assay (RIPA) was developed using a 336-amino-acid segment of GBV-C E2 produced using the Semliki Forest virus (SFV) expression system and grown in BHK-21 cells (A.T.C.C. CCL-10) as previously described [Pilot-Matias et al., 1996b].

Specimens

Blood donors. Serum samples were obtained from volunteer blood donors in the U.S. with normal serum alanine aminotransferase (ALT) levels (<45 IU/L) and considered to be at low risk for infection with GBV-C. These specimens were negative for hepatitis B surface antigen (HBsAg), antibodies to hepatitis B core (anti-HBc) and antibodies to hepatitis C virus (HCV, 2nd generation assay). All tests are commercially available from Abbott Laboratories, North Chicago, Illinois.

Commercial plasmapheresis donors. Plasma was available from commercial plasmapheresis donors in the U.S. These specimens were negative for HBsAg and antibodies to HCV, (2nd generation assay).

IVDUs. Specimens were obtained from IVDUs, a population considered at high risk for exposure to parenterally transmitted agents. Seroprevalence for antibodies to HCV (99%) and hepatitis B virus (HBV) (75%) were previously reported [Dawson et al., 1996]. Serial bleeds were available from 21 of these IVDUs. The span of time under evaluation ranged from 13 to 39 months past the first available bleed date.

Hepatitis patients. Specimens from patients diagnosed with acute or chronic non A-E hepatitis and acute or chronic HCV were previously described [Dawson et al., 1996]. Serial bleeds were available from 6 patients diagnosed with chronic HCV, 3 patients diagnosed with acute HCV and 1 patient diagnosed as chronic non A-E hepatitis. Serial bleed specimens were also available from 1 transfusion recipient diagnosed with hepatitis after receiving GBV-C RNA positive blood.

RESULTS

Prevalence of GBV-C

Blood donors. In a population of 199 volunteer blood donors with normal ALT values, 9 (4.5%) were

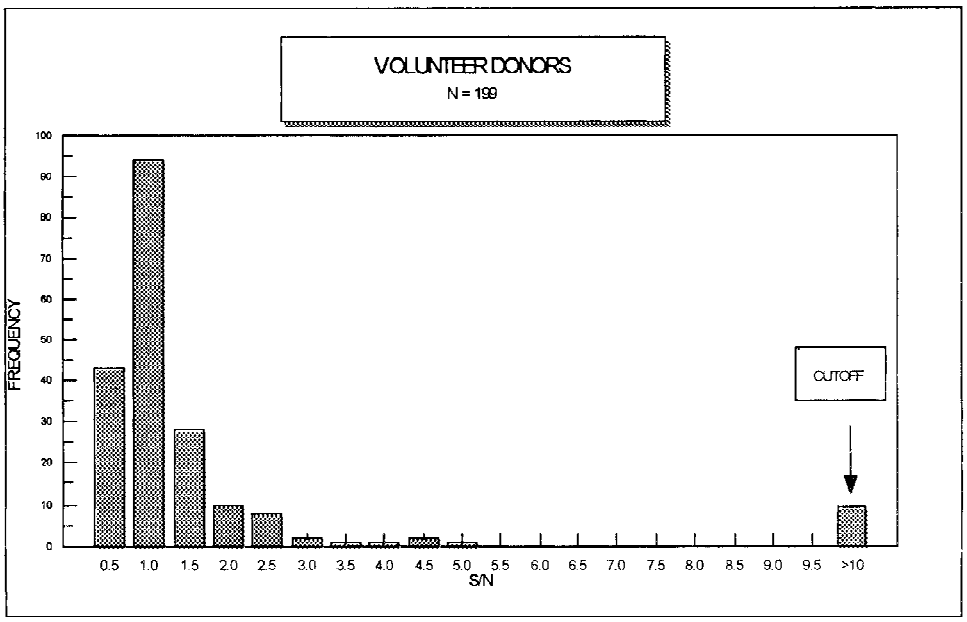

Fig. 1. Distribution of signal to negative control (S/N) values of 199 volunteers donors tested in the GBV-C E2 ELISA.

TABLE I. Summary of GBV-C Prevalence Studies

Category	Number positive (% of total) GBV-C RNA	Antibody to GBV-C E2	Total prevalence[a]
Volunteer donors (n = 199)	3 (1.5)	9 (4.5)	11 (5.5)
Commercial donors (n = 711)	93 (13.1)	195 (27.4)	288 (40.5)
Acute non A–E hepatitis (n = 51)	4 (7.8)	18 (35.3)	22 (43.1)
Chronic non A–E hepatitis (n = 41)	3 (7.3)	14 (34.1)	17 (41.5)
Acute HCV (n = 41)	8 (19.5)	22 (53.7)	29 (70.7)
IVDUs[b] (n = 102)	15 (14.7)	76 (74.5)	91 (89.2)

[a]Total prevalence is measured as the sum of RNA positive and ELISA positive specimens, expressed as no. positive (% of total).
[b]IVDUs: Intravenous Drug Users.

positive for antibody to GBV-C E2 when tested in the GBV-C E2 ELISA (Table I). All 9 samples were tested by RIPA and were confirmed as reactive. These specimens were removed from the calculation of the population mean and standard deviation. The distribution of the normal population is shown in Figure 1. There were a total of 3 (1.5%) volunteer blood donor samples which were positive for GBV-C RNA, one of which was also positive for antibody to GBV-C E2. Thus, the incidence of GBV-C exposure in volunteer blood donors is 5.5%.

Commercial plasmapheresis donors. Among commercial plasmapheresis donors, 93 of 711 (13.1%) were positive for GBV-C RNA and 195 (27.4%) were reactive for antibody to GBV-C E2 (Table I). There were no samples that contained both GBV-C RNA and antibody to GBV-C E2 in this group. The total exposure among plasmapheresis donors is 40.5%.

Non A-E hepatitis. Of the 51 specimens diagnosed with acute non A-E hepatitis, 4 (7.8%) were positive for GBV-C RNA and 18 (35.3%) were reactive for antibody to GBV-C E2 (Table I). Similarly, in the chronic non A-E hepatitis group, 3 of 41 (7.3%) samples were positive for GBV-C RNA and 14 (34.1%) were reactive for antibody to GBV-C E2. Thus, the exposure to GBV-C in acute and chronic non A-E hepatitis specimens is 43.1% and 41.5%, respectively. There were no specimens found to be both GBV-C RNA positive and GBV-C E2 antibody positive in either group.

Acute HCV. In patients diagnosed with acute HCV, 8 of 41 (19.5%) were GBV-C RNA positive while 22 (53.7%) were GBV-C E2 antibody positive. One specimen was positive for both GBV-C RNA and GBV-C E2 antibody. Exposure to GBV-C in this population was 70.7% (Table I).

IVDUs. In a population of intravenous drug users, 15 of 102 (14.7%) were positive for GBV-C RNA and an additional 76 (74.5%) were GBV-C E2 antibody positive for a total exposure to GBV-C of 89.2% (Table I).

GBV-C Status Over Time

The clinical course of a patient who was transfused with GBV-C RNA positive blood is shown in Figure 2. This patient received blood from 7 donors; sera from six of these donors was available for testing, one of which was GBV-C RNA positive by RT-PCR. This patient was not coinfected with HCV; results from HCV PCR performed on specimens from the day prior to transfusion and 78 days post-transfusion were negative. Serial bleed specimens were tested for antibody to GBV-C E2 as well as GBV-C RNA. In addition, serial ten-fold di-

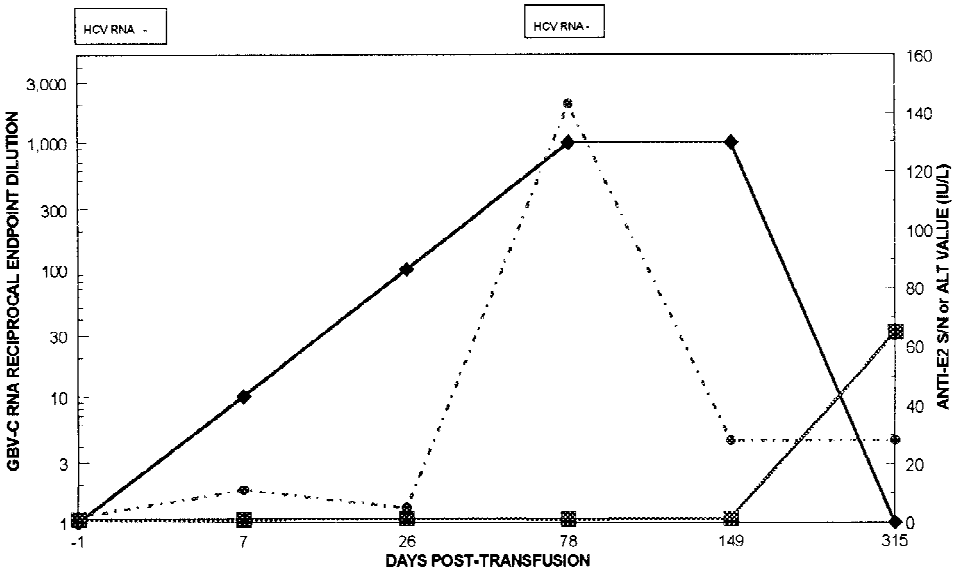

Fig. 2. Patient with transfusion acquired GBV-C. Reciprocal endpoint dilution of GBV-C RNA as measured by RT-PCR/OH (diamonds), GBV-C E2 ELISA S/N (squares) and levels of alanine aminotransferase (ALT; dashed line) are shown plotted against time in days post-transfusion in a patient infected only with GBV-C. Qualitative results for HCV are shown (boxed) above the time points tested (negative, minus sign).

lutions were performed on extracted RNA from all samples and run by RT-PCR/OH as a measure of the magnitude of viremia. This patient became GBV-C RNA positive 7 days after transfusion and continued to be viremic through day 149 post-transfusion with an increase in GBV-C RNA titer of approximately two orders of magnitude between days 7 and 78. ALT elevations were mild, peaking at 143 IU per liter at day 78. At day 315 post-transfusion, the patient became reactive for antibody to GBV-C E2 while GBV-C RNA was no longer detectable. In this case, the immune response to GBV-C E2 correlated with viral clearance.

Serial bleeds were available from 21 IVDUs, the time span under evaluation ranged from 13 to 39 months past the first available bleed date. A total of 7 patients (33.3%) were GBV-C RNA positive on the first available bleed date (Table II) and remained viremic throughout the course of their evaluation. The magnitude of viremia, depicted as reciprocal endpoint dilution, was measured in serial bleeds available from these patients (Fig. 3). All 7 IVDUs remained persistently infected with GBV-C for 23 months or greater, and displayed a fairly narrow range of variability with respect to endpoint titer of GBV-C RNA over time, varying at most by two orders of magnitude.

The remaining fourteen IVDUs (66.7%) for which serial specimens were available, were GBV-C E2 antibody positive on the first bleed date and remained so

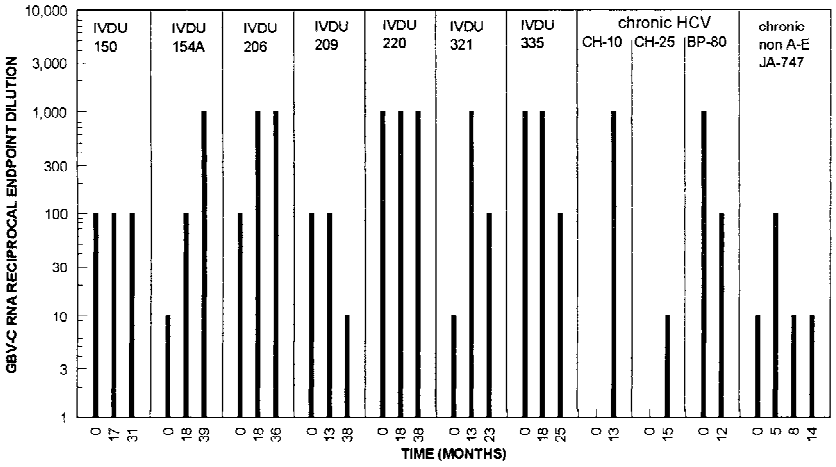

Fig. 3. Titration of GBV-C RNA positive IVDU and hepatitis patients. Reciprocal endpoint dilutions as measured by RT-PCR/OH performed on serial bleeds from all RNA positive IVDU and hepatitis patients. Each patient is shown plotted against time in months past first available bleed date.

TABLE II. Serologic Status in Serial Bleeds of Persistently Infected GBV-C RNA Positive Patients

Patient category and no.	GBV-C RNA status	GBV-C E2 antibody reciprocal endpoint dilution		
		Time 0	10–18 months	27–39 months
IVDUs				
150	+	–	–	–
154A	+	–	–	–
206	+	–	–	–
209	+	–	–	–
220	+	–	–	–
321	+	–	–	NT
335	+	–	–	NT
Chronic HCV				
CH-10	seroconverts to RNA +	–	–	NT
CH-25	seroconverts to RNA +	–	–	NT
BP-80/BP-93	+	–	–	NT
Chronic non A–E				
JA-747	+	–	–	NT

NT: Sample not available for testing.

throughout the course of their evaluation (Table III). Serial dilutions from each bleed date were tested in the GBV-C E2 ELISA and demonstrate the constancy of antibody response within each patient, varying at most by 1 two-fold dilution over time. In contrast, antibody titers between patients varied greatly and ranged from 336 to 21,504.

In hepatitis patients, serial bleeds were available from 11 to 29 months past the first available bleed date. Of the 6 chronic HCV patients evaluated, a total of 3 (50%) were GBV-C RNA positive (Table II); two of these became viremic during the course of evaluation. The remaining three (50%) were GBV-C E2 antibody positive with endpoint titers that ranged from 672 to 5,376 (Table III). In patients with acute HCV, 3 of 3 (100%) were positive for antibody to GBV-C E2 with endpoint titers that ranged from 672 to 1,344 (Table III). One patient diagnosed with chronic non A-E hepatitis was GBV-C RNA positive over a period of 14 months (Fig. 3). The endpoint titer within each GBV-C E2 antibody positive hepatitis patient remained constant over time. In addition, the GBV-C RNA positive hepatitis patients, evaluated for overall magnitude of viremia, showed reciprocal endpoint dilution titers in the same range as those seen in the IVDU patients (Fig. 3).

DISCUSSION

The seroprevalence of GBV-C was underestimated in studies using procaryotically expressed regions of the GBV-C genome as the antigenic targets in ELISAs [Dawson et al., 1996; Pilot-Matias et al., 1996b], as well as in studies using only RNA positivity as a measure of exposure [Dawson et al., 1996]. Recently, an ELISA using mammalian cell-expressed GBV-C E2 protein as its antigenic target has been developed and demonstrates the utility of this protein in determining exposure to GBV-C [Dille et al., 1997]. Patients who seroconvert for antibody to GBV-C E2 remain negative when tested against the procaryotically expressed proteins from GBV-C. To date, E2 appears to be the only predominantly immunoreactive region of GBV-C. It is apparent from the data presented above that testing for both antibody to GBV-C E2 and GBV-C RNA is necessary to determine exposure to GBV-C.

The current study verifies and expands earlier observations that segregates GBV-C exposed individuals into two categories; RNA positive or E2 antibody positive [Pilot-Matias et al., 1996b; Dille et al., 1997]. Of the 1,145 specimens evaluated in this study by both RT-PCR and ELISA, a total of 458 (40%) have been exposed to GBV-C, of which only 2 (0.4%) were positive for both GBV-C RNA and GBV-C E2 antibody. These samples may have been obtained at their transition point where both antibody to GBV-C E2 and GBV-C RNA are co-detected for a short period of time or are from patients that are chronic carriers. In every other case, a specimen was either RNA positive or antibody positive indicating an apparent mutual exclusivity with respect to these two parameters, unlike that seen in patients actively infected with HCV where a positive correlation exists between detection of RNA and antibody to E2 [Lesniewski et al., 1995]. Viral clearance was also evident in the GBV-C transfusion patient where viremia became undetectable upon the detection of GBV-C E2 antibody. Thus, it appears that once an individual mounts an antibody response to GBV-C E2, virus is cleared from the system or falls below currently detectable levels. This observation lends support to the

TABLE III. Serologic Status in Serial Bleeds of GBV-C RNA Negative Patients

Patient category and no.	GBV-C RNA status	GBV-C E2 antibody reciprocal endpoint dilution		
		Time 0	10–18 months	27–39 months
IVDUs				
3	—	336	336	336
10	—	2,688	2,688	2,688
12	—	336	NT	336
50	—	2,688	2,688	2,688
61	—	5,376	5,376	NT
61A	—	672	NT	672
72	—	21,504	21,504	21,504
126	—	21,504	10,752	10,752
140	—	2,688	2,688	2,688
153	—	672	672	NT
178	—	336	336	336
215	—	2,688	2,688	NT
219	—	21,504	21,504	NT
335A	—	5,376	5,376	NT
Chronic HCV				
DO-286/CH-11	—	5,376	5,376	NT
MJ-678	—	672	672	NT
CH-94	—	2,688	2,688	NT
Acute HCV				
CS-721	—	1,344	1,344	NT
DS-611	—	1,344	NT	1,344
CG-587	—	672	672	NT

NT: Sample not available for testing.

idea that antibody to GBV-C E2 is a marker of viral clearance [Pilot-Matias et al., 1996b].

The total exposure to GBV-C in a population of U.S. volunteer blood donors is 5.5%; approximately half that found by Tacke et al. [1997], who used both an HGV E2 ELISA and HGV RT-PCR to measure exposure in a similar group in Germany. Either estimate of exposure to GBV-C, however, is approximately 10-fold higher than that reported for HCV in volunteer blood donors (0.5%) [Kleinman et al., 1992]. Why exposure to GBV-C is so high in presumably healthy, low risk populations is unknown. In commercial donors, exposure to GBV-C is similar to that observed in commercial plasma pools by Nubling et al. [1996]. Again, drawing a comparison with HCV, the prevalence of GBV-C is approximately 40-fold higher than that reported for HCV among commercial plasma donors [Lee et al., 1995]. The high exposure rate to GBV-C observed in the population of IVDUs (89.2%) is similar to that observed by Tacke et al. [1997], and approaches prevalence reported for HCV (99%) [Dawson et al., 1996]. These data support previous observations that GBV-C is effectively transmitted through parenteral routes [Dawson et al., 1996; Linnen et al., 1996; Schleicher et al., 1996].

The prevalence of GBV-C as determined by testing for GBV-C E2 antibody is 2 to 5 fold higher than that of RNA in each of the populations studied, again indicating that the true measure of exposure to GBV-C is underestimated when only RNA testing is performed. These data also suggest that more than half of the individuals who become infected with GBV-C are able to clear the virus. One might expect the ratio of antibody to RNA to be reversed when GBV-C is associated with an active disease, since presumably in that population the prevalence of protective antibody should be lower than in individuals who have no evidence of active disease.

Serial bleeds from 31 patients were used to evaluate persistence of GBV-C viremia and duration of antibody response. During the span of time under evaluation, every patient who was RNA positive on the first available bleed date remained viremic throughout the course of study, often more than 3 years. Thus, infection with GBV-C can be fairly long-lived in individuals who do not mount an immune response to GBV-C. Similarly, all patients who were antibody positive on the first bleed date remained so throughout the course of study, again, some greater than 3 years. It is worth noting that in the population of IVDUs, persistence of GBV-C RNA could be due to continual reinfection in combination with immunosuppression due to coinfection with other viruses [Jarvis et al., 1996], or perhaps the ability of GBV-C to evade immune surveillance [Dille et al., 1997].

Titration studies were used to evaluate the magnitude and variation of both viremia and antibody titer over time. The results demonstrate the constancy of the GBV-C E2 antibody response and the persistence of viremia with some variability in viral load. Overall, very little change was seen in the serologic profile of GBV-C exposed individuals within the time frame studied.

Further longitudinal studies would be useful in determining how these markers of GBV-C exposure change over time. Existing data suggest that antibodies to GBV-C E2 play a role in viral clearance. However, it is unclear whether antibodies to GBV-C E2 are protective against infection with GBV-C.

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